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Title: Chemotherapeutic and Nontarget Side-effects of Benomyl to the Orange Tortrix (*Argyrotaenia citrana*) and the Braconid Endoparasite *Apanteles aristoteliae*.

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Benomyl, a systemic benzimidazole fungicide was incorporated into artificial diet and evaluated for its oral toxicity to nonparasitized and parasitized third instar orange tortrix larvae, *Argyrotaeniae citrana* (Fernald) and its food chain toxicity via the host to the solitary braconid endoparasite *Apanteles aristoteliae* Vierek.

Concentrations of benomyl $\leq$ 300 ppm were sublethal to nonparasitized hosts. Increasing concentrations of benomyl resulted in significant decreases in the percent of nonparasitized larvae pupating.

Parasitized hosts feeding on treated diet containing 300 ppm benomyl or its principal metabolite MBC, after parasitization, had significantly greater ($P \leq 0.05$) percentages of host larvae pupating and surviving to

adulthood than controls. This chemotherapeutic effect was equivalent for hosts feeding on 300 ppm benomyl treated diet for only the first 24 hours after parasitization as compared to hosts feeding continuously. Increasing concentrations of benomyl resulted in decreased percentages of parasitized hosts pupating and increased percentages of hosts blocked in their larval development. Comparisons of parasitized and nonparasitized larval mortalities directly due to benomyl indicated that parasitized larvae (LC50 = 2151 ppm) were less susceptable than nonparasitized larvae (LC50 = 806 ppm).

Parasite emergence from hosts was reduced by all fungicide treatments. However, only when hosts fed on benomyl treated media with concentrations $\geq$ 300 ppm and after exposure to parasites was parasite emergence significantly reduced ($P \leq 0.05$). The potential uses of benomyl or like substances to study parasite-host relationships and how benomyl may impact on field populations of these insects are discussed.

Chemotherapeutic and Nontarget Side-effects of Benomyl to the Orange Tortrix (*Argyrotaenia citrana*) and the Braconid Endoparasite *Apanteles aristoteliae*.

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INTRODUCTION

Many agricultural pesticides disrupt control of important arthropod pests by influencing their natural enemies. More specifically, the destructive effects of insecticides and acaracides on arthropod predator and parasite populations are well documented (Croft & Brown 1975). They include such diverse responses as direct mortality, sublethal influences on reproduction, development, longevity, and behavior and resistance development by these species (Croft 1977). Pesticides can indirectly cause pest resurgence and secondary pest outbreaks due to temporary elimination of the natural enemies' food supply. This may lead to natural enemy starvation, emigration and or lapses in reproduction (Flint & Van den Bosch 1981).

Herbicides and fungicides also have similar nontarget effects as insecticides on natural enemies (Hassan et al. 1983). Of the fungicides, the benzimidazoles are the most prevalently reported. Benomyl (Benlate) [(R)1], a carbamate benzimidazole, causes direct toxicity to the predacious mite *Amblyseius fallacis* Garman (Nakashima & Croft 1974),

a direct toxicity to the coccinellid *Stethorus punctum* Leconte (Coburn & Asquith 1973), oviposition repellancy to the white fly parasite , *Encarsia formosa* Gahan (Irving & Wyatt 1973) and reduced emergence of the parasites *Micropletis croceipes* (Cresson) and *Cotesia marginventris* (Cresson) from the host, *Heliothis zea*, (Horton et al. unpublished and Teague et al. 1985).

These reports of nontarget effects by benzimidazole fungicides to arthropod natural enemies prompted this study. My objective was to evaluate the effects of benomyl and MBC, (Methyl-2-benzimidazole carbamate, a breakdown product of benomyl) as food chain toxicants to the solitary braconid endoparasite (*Apanteles aristoteliae* Vierek) via its host, the orange tortrix (*Argyrotaenia citrana* Fernald). During experiments, hosts were reared on artificial diet incorporated with sublethal levels of benomyl to the host. Moth larvae were exposed to different concentrations of the toxicant and for different periods of time before and after exposure to parasites. Comparative tests were made on both nonparasitized and parasitized hosts. The specific responses of hosts and parasites to benomyl and MBC measured in these tests included altered developmental times, increased or reduced survivorship, and developmental anomalies.

LITERATURE REVIEW

This review contains four sections: 1) the general nonselective nature of pesticides and an evaluation of beneficial arthropod responses to pesticides, 2) the fungicide benomyl and related benzimidazoles, and their effects on target and nontarget organisms including uses and possible modes of action, 3) the biology and natural history of the lepidopteran host, *Argyrotaeniae citrana* Fernald, and 4) similar information on the braconid parasite, *Apanteles aristoteliae* Vierik.

The nonselective nature of pesticides. In 1980, Metcalf declared "The Era of Integrated pest Management" (IPM) to have begun in 1976. He of course was speaking of the implementation of IPM, since many publications prior to 1976 discussed this concept in detail (e.g. van den Bosch & Stern, 1962). What IPM means to this author is that suggested by Franz (1960): the broadest embrace of all pest control measures along with maximum use of cultural and biological controls. Implicit in IPM is maximum use of natural enemies of arthropod pests (predators, parasites and pathogens) supplemented by selective pesticides (Croft and Brown, 1975).

Although the IPM concept is sound, its implementation often is not possible due to the lack of specificity present in conventional insecticides, herbicides and

fungicides. Many of these compounds not only affect target organisms including insects, plants or fungi, respectively, but other species in different classes and phylla. This lack of specificity is manifested specifically in nontarget effects on beneficial arthropod predators and parasites. For insecticides, reviews of these effects are found in Ripper (1956), Bartlett (1964) and Croft and Brown (1975). More recent data for herbicides and fungicides are also available in Franz et al. (1974), Hassan (1974), Tanke and Franz (1978), Franz et al. (1980) and Hassan et al. (1983).

In determining the selectivity of pesticides, it often is necesary to evaluate each relationship specifically for a given pesticide, arthropod and natural enemy. In part, this is due to the great seasonal, geographical and biological variation in arthropod hosts and their natural enemies (Bartlett 1964). Biological characteristics also may affect the susceptibility of a species to a pesticide. These include, whether the organism is a free living or host associated form of natural enemy (i.e. predator or adult parasite versus larval endo or ectoparasite), its physiological condition (starved or dehydrated), its developmental stage tested, its age, size, or if an adult or larva, its sex (Croft 1977).

An organized program for evaluating effects of pesticides on beneficial arthropods was begun in 1974 in Europe with the formation of the working group "Pesticides and Beneficial Arthropods" by the International Organization for Biological control (IOBC) Western Palaeartic Regional Section (WPRS) (Franz et al. 1980). In addition to stimulating greater initiatives for testing nontarget effect of pesticides, several important conclusions were reached by this group after evaluating the influence of 40 pesticides on 13 different beneficial arthropods. First, only a few pesticides can be considered harmless to beneficial arthropods. Second, the mode of exposure and stage of the predator or parasite is critically important in evaluating the effects of a pesticide. Third, fungicides and herbicides as well as insecticides and acaracides can be detrimental to beneficial arthropods. Lastly, different beneficial species are affected differently by different pesticides (Franz et al. 1980, Hassan et al. 1983). Each of these points is similar to those made earlier.

Fungicidal effects on nontarget pests and beneficial species. As early as 1956, Ripper recognized that many fungicides in general use were insecticidal to beneficial arthropods. Unfortunately very little work was done to evaluate the interactions of fungicides and beneficial arthropods specifically. Recently, the benzimidazole

fungicides have been reported as having nontarget effects to predators and parasites. Benomyl, one member of this group of compounds, influences a wide range of organisms, including target plant diseases caused by pathogenic fungi (Dekker 1977), a number of annelid, nematode, arthropod pests (Table 1), several entomophagous fungi and a diversity of arthropod natural enemies (Table 2).

Benomyl's potential as a general cytological or broad spectrum poison was demonstrated by Styles and Garner (1974) in mammary tissue cultures. Other benzimidazoles have demonstrated an ability to act as broad spectrum toxicants. For example, four aquatic organisms, an alga (*Chlorella pyranoidosa*), a crustacean (*Daphnia magna*) and two species of fish (*Lebistes reticulatus* and *Salmo gairdneri*), responded with EC50 (effective concentrations of 50% mortality) values in the ranges of 0.48-3.7, 7.8-130 and 0.34-1.8 ppm for benomyl, methyl thiophanate (MT) and MBC respectively (Canton 1976).

Benomyl and several related compounds are toxic to a number of nonfungal pests (Table 1). Earthworms, *Lumbricus terrestris* (Linneaus) in particular, are sensitive to several benzimidazole compounds (Stringer & Lyons 1974, Stringer & Wright 1976) which has led to their use to selectively eliminate populations of earthworms near airports (Tomlin et al. 1981). Conversely benomyl

Table 1. Nontarget effects of the benzimidazole fungicides to nonfungal pest organisms including worms, nematodes, mites and insects.

CMPD NAME[1]	CONCENTRATION or RATES[2]	AFFECTED ORGANISM Species/group	EFFECTS	REFERENCE
B, MT	1.4kg/ha	*Lumbricus terrestris*/ Annelida	extinction in ag. systems	Stringer & Lyons 1974
B, MBC, F, TB	0.00056mg per worm	*L.terrestris*/ Annelida	chronic oral toxicity highly toxic	Stringer & Wright 1976
B	2.24kg ai/ha	*L.terrestris*/ Annelida	85% reduction in population	Tomlin etal 1980
B	67ppm	*Heterodera avenae*/ Nematoda	inhibited cyst formation & root invasion	Cook & York 1972
B, MBC	0.25mg/ml	*Acyrthrosiphon pisum*/ Aphidae on bean, *Vicia fabae*	systemic toxicity	Partiss & Bailiss 1980
AB, TB, Z	0.25mg/ml	*A.pisum*/ Aphidae on bean, *V. fabae*	no effect	Partiss & Bailiss 1980
B MBC	0.25mg/ml	*Aphis fabae* & *A.pisum*/ Aphidae on bean, *V. fabae*	systemic toxicity & reduced fecundity	Bailiss et al. 1978

Table 1 (continued)

Nontarget effects of the benzimidazole fungicides to nonfungal pest organisms.

CMPD NAME[1]	CONCENTRATION or RATES[2]	AFFECTED ORGANISM Species/group	EFFECTS	REFERENCE
B	not available	*Microsiphum avenae* & *Rhopalosiphum padi*/ Aphidae	systemic toxicity,	Hinz & Daebler 1973
B	10,900ppm	*Heliothis zea*/ Noctuidae	food toxicant 20% mortality	Livingston et al. 1978a
B	3080ppm	*Psuedoplusia includens*/ Noctuidae	food toxicant 50% mortality	Livingston et al. 1978a
B	1869ppm	*Trichoplusia ni*/ Noctuidae	food toxicant 50% mortality	Livingston et al. 1978a
B	2g/3.8 liters media	*H.zea*/ Noctuidae	no effect	Bell et al. 1981
B	2g/3.8 liters media	*H.zea*/ Noctuidae	mold inhibitor in artificial media	Bell et al. 1981
B	0.03% ai	*Tetranychus urticae*/ Tetranychidae	ovicidal & trans-ovarial	Spaddafora & Lindquist 1972
B	4-16% ai per 100gal	*Tetranychus pacificus*/ Tetranychidae	ovicidal & trans-ovarial	Stafford & Fukushima 1971

Table 1 (continued)

Nontarget effects of benzimidazole fungicides to nonfungal pest organisms.

CMPD NAME[1]	CONCENTRATION or RATES[2]	AFFECTED ORGANISM Species/group	EFFECTS	REFERENCE
B	20-100mg per plant	*T.urticae*/ Tetranychidae	ovicidal & trans-ovarial	Binns 1969
B	<100ppm	*T.urticae* *Panonychus ulmi*/ Tetranychidae *Phyllsoptruta oleivora*/ Eriophyidae	ovicidal	Delp & Klopping 1968

1:Compound names; B=Benomyl, MT=Methyl Thiophanate, TB=Thiabendazole,F=Fuberidazole, AB=aminobenzimidazole, BZ=Benzimidazole, MBC= Methyl benzimidazole carbamate

2. Abbreviations used. a.i.= active ingredient; ha = hectare; gal.= gallons; ppm = parts per million

can decimate earthworm populations in agriculture which reduces soil aeration and soil building activity (Stringer & Lyons 1974). Benomyl's toxicity also extends to nematodes. Soil drenches as low as 67 ppm prevent root invasion and cyst formation by, *Heterodera avenae*, and increased dry season mortalities of this pest (Cook & York 1972).

Benomyl and MBC have important aphicidal properties. Several species are susceptible to soil drenches and to a lesser degree, foliar applications (Table 1). The effects from feeding on systemically treated plant tissues are direct mortality, reduced fecundity and in some cases a behavioral preference for untreated versus treated leaves (Parr & Binns 1969, Binns 1969, Hinz & Daebler 1973, Bailiss et al. 1978, Partis & Bailiss 1980).

In contrast, benzimidazoles are relatively nontoxic to some lepidoptera. Benomyl and thiabendazole, when evaluated against four species on soybean, were toxic only at application rates 1-2 orders of magnitude (3000-10900ppm) greater than the recommended field rates (300-600ppm) (Table 1; Livingston et al. 1978a). Bell et al. (1981) confirmed this relatively low toxicity to *Heliothis zea* (Boddie) and recommended the use of benomyl as a mold inhibitor in artificial media for insect rearing.

Benomyl's acaricidal activity has been demonstrated against the two spotted spider mite, *Tetranychus urticae* (Koch) (Spadafora & Lindquist 1972, Boykin & Campbell 1982), pacific spider mite, *T. pacificus* (McGregor) (Stafford & Fukushima 1970), European red mite, *Panonychus ulmi* (Koch) and citrus rust mite, *Phyllocoptruta oleovora* (Ashmead) (Delp & Klopping 1968). The effects include a systemic toxicity due to feeding on plant tissues, transovarial and ovicidal activity when gravid females feed on treated plant tissues, decreases in fecundity, longevity and viability of eggs from direct and residual contact (Table 1).

Without argument, these systemic and contact nematicidal, acaricidal, aphidicidal and fungicidal properties of benomyl give it a unique broad spectrum usefulness on certain crops.

Benomyl also has sublethal and lethal insecticidal activity on nontarget beneficial organisms (Table 2). As might be expected, a major group affected is entomophthoraceous fungi. Benomyl reduces the abundence of several species attacking the bean aphid, *Aphis fabae* Scop. (Table 2; Wilding 1981). The dominant species, *Erynia neophidis* Remaud & Henn., was the most noticeably decreased in abundance. The impact was minimized if applications were made when the fungi were actively

Table 2. Nontarget effects of benzimidazole fungicides on nontarget beneficial organisms

CMPD NAME[1]	CONCENTRATION or RATES[2]	AFFECTED ORGANISM Species/group	EFFECTS	REFERENCE
B	559g 50wp/ha	*Nomureae rileyi*/ Entomogeneous fungi on lepidopteran hosts: *Plathypena scabra* (F), *Psuedoplusia includens* (Walker) *Heliothis* spp. & *Anticarsia gemmatalis* (Hubner)	reduced incidence delayed onset of epizootic phase	Horton et al. 1980
B, TB	0.28-1.12kg ai per ha	*N. rileyi* & *Entomophthora gammae* Entomogeneous fungi on lepidopteran hosts: *P. scabra*, *P. includens* & *A. gemmatalis*	no effect	Livingston et al. 1978
B, TB	0.28-1.12kg	*Nabis* spp./ Nabidae *Geocoris punctipes* (Say)/ Geocorinae *Coleomegilla maculata* (Degeer)/ Coccinelidae	no effect	Livingston et al. 1978
B	0.6kg ai per 340 litre per ha	*Erynia neophadis*/ Entomorphthoracae on aphid host *A. fabae*	reduced aphid mortality	Wilding 1981

Table 2 (continued)

Nontarget effects of benzimidazole fungicides on beneficial organisms

CMPD NAME[1]	CONCENTRATION or RATES[2]	AFFECTED ORGANISM Species/group	EFFECTS	REFERENCE
B	2-6oz. per 100gal	*Amblyseius fallacis*/ Acarina; Phytoseiidae	direct & food chain toxicity	Nakashima & Croft 1974
B	20-100mg per plant	*Phytoselius* spp./ Phytoseiidae	reduced egg viability	Parr & Binns 1970
MBC	0.05% ai	*Amblyseius potentillae* & *Phytoseilius persimilis*/ Phytoseiidae	direct & residual toxicity	Hassan et al. 1983
MT	0.1% ai	*A.potentillae* Phytoseiidae *Syrphus vitripennis*/ Syrphidae	direct & residual toxicity	Hassan et al. 1983
B	0.3g/litre	*Euseius hibisci*/ Phytoseiidae	secondary pest outbreaks	Ball 1982
B	0.25lbs per 100 gals	*Stethorus punctum*/ Coccinelidae	50% mortality	Coburn & Asquith 1973
B	0.025% ai	*Encarsia formosa*/ Aphelinidae on whitefly host *Tiarleurodes vaporariorum* (Westwood)/ Aleyrodidae	oviposi-tional repellant	Irving & Wyatt 1973

Table 2 (continued)

Nontarget effects of benzimidazole fungicides on beneficial organisms

CMPD NAME[1]	CONCENTRATION or RATES[2]	AFFECTED ORGANISM Species/group	EFFECTS	REFERENCE
B	EC50 7.0-8.4ppm	*Cotesia marginventris/* Braconidae on Lepidopteran hosts:*H. zea, P. includens, Spodoptera exiguae* (Hubner) & *S. ornithogalli* (Guenee)	food chain toxicity & reduced larval emergence	Teague et et al. 1985
B	EC50 7-9ppm	*Micropletis croceipes/* Braconidae Lepidopteran host *H. zea*	food chain toxicity & reduced larval emergence	Horton et al. unpub.
TB	EC50 3-4ppm	*Micropletis croceipes/* Braconidae	food chain toxicity & reduced larval emergence	Horton et al. unpub

1:Compound names; B=Benomyl, MT=Methyl Thiophanate, TB=Thiabendazole,F=Fuberidazole, AB=Aminobenzimidazole, BZ=Benzimidazole, MBC= Methyl Benzimidazole Carbamate

2. Abbreviations used. wp = wettable powder; ha = hectare; a.i.= active ingredient; EC50 = concentration producing effects in 50 % of the population.

spreading. The influence of benomyl and thiabendazole was evaluated on three lepidopteran soybean pests, and their natural enemies including predators, parasites and two species of entomophthoraceous fungi (Table 2). No decrease in abundance of predators or parasites or rates of infection by entomophthoraceous fungi were found (Livingston et al. 1978). In contrast, Horton et al. (1980) found at comparable rates of application, in a similar system, the incidence of fungal infection was weakened and epizootic onset delayed.

The evaluation of nontarget effects benzimidazoles on predators has largely been restricted to acarines although Hassan et al. (1983) has recently expanded this list to include several insect predators and parasites. Effects on predacious acarines are basically the same as for phytophagous species. Foliar sprays of benomyl cause direct, residual and food chain toxicity to all life stages of several predatory mite species (Table 2).

When *Phytoseilius* sp. were allowed to feed on prey reared on treated plant tissues, a food chain toxicity resulted, causing gravid female sterility and an ovicidal activity (Parr & Binns 1970). Nakashima and Croft (1974) tested benomyl against *Amblyseius fallacis* (Garman) and found similar results as well as reduced fecundity due to

food chain toxicity. In a field trial evaluating the toxicity of various pesticides on the mite population complex in pecan orchards, benomyl caused immediate reductions of all species, a shift in relative species abundances and secondary pest outbreaks due to elimination of the predatory mite, *Euseius hisbisci* (Chant) (Ball 1982). In direct and residual toxicity tests, MBC was extremely toxic to *Amblyseius potentillae* Garman and highly toxic to *Phytoselius persimilis* (Athias-Henriot) (Hassan et al. 1983). MT was moderately toxic to *Syrphus vitripennis* and extremely toxic to *A. potentillae* (Hassan et al., 1983). Another predator to which benomyl is moderately toxic by contact is the coccinllid *Stethorus punctum* (Le Conte), (Colburn and Asquith 1973). However, the upper rate tested (300 ppm) was equivalent to the minimum suggested field rate, producing a 50% mortality.

Encarsia formosa (Gahan), a hymenopteran parasite of the glasshouse whitefly, was one of the first parasitoids to be tested for toxicity of benomyl. Adults were repelled by residues of benomyl on both scales and substrate (Irving and Wyatt 1973). In contrast to the low toxicity of benomyl and thiabendazole to soybean pest lepidoptera larvae (Table 1, Livingston et al. 1978a), these compounds have a high food chain toxicity to their principal endoparasites, *Microplitis croceipes* (Cresson) and *Cotesia marginiventris* (Cresson). Concentrations of 4

and 10 ppm, benomyl and thiabendazole respectively, in media reduced *M. croceipes* emergence from *H. zea* larvae by 50% (EC50) (Table 2). Although emergence from the host by the parasite was effected, treatments did not affect the parasite's ability to kill the host (Horton et al., unpublished). With the parasite, *C. marginventris*, developing on four lepidopteran pests soybean fed on media incorporated with benomyl, similarly low Ec50 values (7-8.4 ppm) for larval parasite emergence from three of the four hosts were observed (Table 2, Teague et al. 1985). However, in *Spodotera ornithogalli* (Guenee), a species not included in the Horton study, increasing benomyl concentrations had no effect on larval parasite emergence. Of particular note was the correlation of increased survival of parasitized hosts, *S. exiguae* (Hubner), with increasing benomyl concentrations. Benomyl seemed to provide a chemotherapeutic benefit to the host by ridding it of its parasite (Teague et al. 1985).

In conclusion, for most [pesticide] <-> [plant] <-> [pest] <-> [natural enemy] systems, the presense or absence of pesticidal side effects can not be extrapolated to other systems. The effects of any given pesticide on any given system must often be evaluated individually for all life stages, utilizing the appropriate modes of exposure and exposure regime. These principals are

especially appropriate for chemicals having such complex effects as benomyl and its benzimidazole relatives.

Benomyl uses, properties and mode of action. Benomyl is a systemic fungicide registered by the DuPont de Nemoirs of Wilmington, Del. for 58 crops (including the important crops of blueberries and caneberries in the Pacific Northwest and grapes, lemons, oranges and grapefruit in California) and 28 pathogens (DuPont 1985). In addition to a broad antifungal activity, original patent applications included antihelminthic activities (Corbett 1974). Recommended rates of application range from 0.5-1.0 pounds of 50WP per 100 gallons of water per acre for most crops (DuPont 1985 and Oregon Plant Disease Handbook 1985). This is equivalent to a 300-600 ppm solution.

The solubilty of benomyl in water is approximately 3.8 ppm at 20^0 C. In water its half life is more than 7 hours (Austin & Briggs 1976). Benomyl exists in a stable equilibrium with Methyl-2-benzimidazole carbamate (MBC) and butyl isocyanate (BIC), with the quantitative reformation of benomyl from MBC on the addition of excess BIC (Chiba and Cherniak 1978).

The loss of fungitoxic activity of benomyl and MBC is promoted by high pH and or intense photoradiation. There is no effect of pH on the fungitoxicity of MBC in the

range of 5-7, however total inactivation of the compound does occur at a pH of 9 (Woodcock 1977).

There is no single mode of action that has accounted for the different benzimidazole compounds toxicity to all organisms. Benomyl, the thiophanates, MBC, and BIC all differ to some degree in their toxicity and range of susceptible organisms. Benomyl and MBC express different levels of fungitoxicity towards different fungi (Hammersclagg & Sisler 1972, Hall 1982). Hammersclagg & Sisler (1973) attributed this to the fungictoxic activity of BIC, since both BIC and benomyl have been characterized to give off a fungitoxic vapor (Helms 1981, Hammersclagg & Sisler 1973, Seigal & Zabbi 1972).

BIC and other related compounds have been identified as inhibitors of induced neoplasias in mammary tissues (Wattenburg 1981). Both benomyl and BIC are capable of inhibiting respiration in certain fungi (Hammersclagg & Sisler 1973). However, MBC is only fungitoxic when susceptible fungi are on a medium capable of supporting growth (Clemmons & Sisler 1971).

Direct information on the mode of action of benomyl and MBC is lacking but supportive data suggests that the principal role is at the level of mitotic inhibition (Davidse 1973) or more specifically inhibition of DNA synthesis (Corbett 1974). The activity may be due to

purine base pair substitution (Corbett 1974) or a spindle fiber poison activity like that of colchicine (Dekker 1977).

Evidence for spindle fiber poison activity was found where MBC binding to certain macromolecules of a susceptible strain of *Aspergillus nidulans* was correlated to that strain's sensitivity to MBC. In addition, the molecular weight of those bound macromolecules was determined to be identical to that of microtubulin protein (Van Tuyl et al. 1974).

The striking resemblance of the benzimidazole ring structure to that of the purine ring structure suggests that the mode of toxicity may be that of base pair substitution resulting in lethal mutations. Several studies have provided supportive data to this hypothesis. Low concentrations of various purines have been found to reduce the toxicity of the benzimidazoles, benomyl, fuberidazole and thiabendazole (Corbett 1974). Also benomyl and its two metabolites MBC and 2-amino-benzimidazole (2-AB) induce low level base pair substitution mutations in *Salmonella typhimurium* (Sieler 1972 & 1973a). Unsubstituted benzimidazole is incorporated into RNA and DNA of *Escherchia coli* (Seiler 1973b) where it replaces guanine (Seiler 1975). These

studies suggest a potentially new type of base pair analogue mutagen (Kappas et al. 1976).

Other possible modes of action which have been explored and discounted include the uncoupling of oxidative phosphorylation, electron transport, and the inhibition of respiration. The last two do occur, but only at concentrations much higher than would account for their antihelminthic activity (Corbett 1974).

Resistance to benomyl in the predatory mite, *Metaseiulus occidentalis* (Nesbitt), has been attributed to elevated Mixed Function Oxidase (MFO) activity (Roush & Plapp, 1982). These results indicate that at least one mode of detoxification is shared with carbamate insecticides.

Inhibition of acetylcholinesterase enzymes by benomyl has been documented. Benomyl and BIC were reported to have *in vitro* acetylcholinesterase activity on the earthworm, *L. terrestris*, brain tissue. However, MBC did not exhibit this activity. Since both benomyl and MBC are equitoxic to this species and acetylcholine levels were normal for *in vivo* brains, it was concluded that the inhibition of acetylcholinesterase was not the mode of action (Stringer & Wright 1976).

Biology and natural history of the host insect; the orange tortrix (*Argyrotaeniae citrana*). Orange tortrix (OT) adults are variable in size, color and markings, depending on developmental conditions and sex. Adults are ca. 10mm long with a 16mm wing spread. Males are smaller than females when reared at the same temperature (Bassenger 1938). Powell (1964) gives a more detailed description of the adults.

Longevity of adult OT was evaluated by Bassenger (1938) for stressed and nonstressed individuals. Under conditions of no food or water longevity was 8-10 days. This time allowed for mating and oviposition of a full complement of eggs. However, when supplied with food and water the life span is extended to 29 days for females and 21 days for males (Bassenger, 1938).

The sex ratio of the OT is approximately 1:1. Adults are sexually mature at eclosion. Although males may mate more than once, Bassenger (1938) felt that under field conditions multiple matings were highly unlikely. Fecundity varies greatly (150-400 eggs per female) depending on size and vigor of females. Adult size and vigor depend principally on temperature and humidity during larval development. Greatest fecundity, size and vigor occurs at 55^{0}F and 70% humidity. Temperatures from of 55-75^{0}F (12.7-23.9^{o}C) produce healthy, vigorous adults (Bassenger 1938).

When temperature and humidity are optimal, eggs are laid in a few (2-3) clusters on any smooth surface. Eggs hatch in 8-20 days depending on the temperature. Although development will occur at extremes of 7.2^{0}C (44.9^{o}F) and 29.4^{0}C (84.9^{o}F), considerable mortality occurs(Bassenger 1938, Coop 1982). The optimum range for egg hatch is 20-28^{0}C (Coop 1982).

Larvae are colored shades of brown to green, 1.5-16mm long and possess 5-7 instars depending on developmental conditions (Bassenger 1938, Coop 1982). Development of larvae occurs from 6^{o}C (43^{o}F) to 32^{o}C (90^{o}F) (Kido etal 1981). The optimum range for development is from 55^{o}-75^{o}F, temperatures above 85^{o}F may cause male sterility. At 75^{o}F and 70% humidity neonate larvae will reach pupation in 20.9 days. However, at 75^{o}F and 35% humidity larvae will take 29 days to reach pupation (Bassenger 1938).

The orange tortrix occurs from Baja California to British Columbia on over 80 host plants. (Bassenger 1938, Johansen & Breaky 1949, Powell 1964, Kido etal 1981). This pest is found on 13 crops, 11 of which benomyl is registered for use on. This insect can cause direct, cosmetic and contaminant damage. Also crop losses occur due to premature fruit drop. In the Pacific Northwest OT is a contaminant in machine picked raspberries.

The effect of natural enemies in keeping orange tortrix populations at below economic thresholds in Washington state was noted by Johansen (1978) and in California orange groves by Bassenger (1938). More recently in California grape and Oregon caneberry, high rates of parasitism have been associated with relatively low OT populations (Kido et al. 1981, Coop 1982). These reports indicate that parasitoid natural enemies may be critically important in keeping the OT populations at low levels.

Biology and natural history of the parasite (*Apanteles aristoteliae* Vierek). Of the 12 primary parasites of the orange tortrix the braconid *A. aristoteliae* has been the dominant species found in most studies (Anonymous 1926, Bassenger 1935 & 1938, Rosenstiel 1949, Coop 1982). *A. aristoteliae* also attacks several other lepidopteran hosts (Krombien 1976) including the spruce budworm, *Choristonuera fumerifera* (Clem), and the strawberry leaf roller, *Anncytus comptana frageria* (W&R).

In Oregon caneberry *A. aristoteliae* can be reared from field collected OT at nearly any time (Coop 1982). It is a solitary endoparasite which exhibits arrhenotokous reproduction. Female adults aggressively attack and parasitize 2-5 instar OT larvae (Bassenger 1938, Coop 1982). There is no difference in developmental rates for

either sex or for individual parasites laid in different sizes of hosts (Bassenger 1938, Coop 1982). At 20°C the developmental time for egg to cocoon is 20.5 days and for cocoon to adult 12.2 days (Coop 1982). Eggs and larvae will develop from 7.2-28°C (Coop 1982).

The longevity of *A. aristoteliae* is as long as 51 days for females and 26 days for males (Coop 1982). Females are receptive to mating and will oviposit in hosts within hours of eclosion. Oviposition by female parasites takes only seconds to complete; hosts show no signs of paralysis after parasitization, although there are a few seconds of excited behavior that follow stinging. There is apparently no host discrimination for previous stinging of the host or for host size. Under laboratory conditions a given host may be stung several times, but only one parasite will emerge and pupate.

Regulation of host development is apparent by *A. aristoteliae*. Smaller hosts continue to gain weight and molt and larger hosts are arrested in their development from oviposition onwards. At the completion of parasite larval development the parasite chews an exit hole at approximately midpoint and dorsal or laterally on the host larva. Once the anterior half of the parasite has emerged the parasite attaches to the host larva with mandibles and begins to feed on the host tissues and fluids. Emergence

is completed after attachment of the mandibles. Consumption of the host is nearly total within a few hours after the initial emergence and the parasite usually leaves only the host head capsule and skin remains. After engorgement on the host larva, a cocoon is spun nearby where pupation occurs.

Emergence from the cocoon involves the adult parasite chewing a circular hole in one end of the cocoon. Food and water are readily accepted at this time. If water is withheld for as short as 48 hrs. premature death of the newly emerged adults may result.

Several characteristics of *A. aristoteliae* make this organism an appropriate subject for pesticide studies. First, it is a dominant natural enemy of OT, and capable of its supression in the field. Secondly, it is widely co-occurrent with OT populations from southern California to British Columbia. This makes *A. aristoteliae* unique compared to other OT natural enemy species which occur in only a portion of the distribution of this pest. Thirdly, the parasite and host can be easily reared in relatively large numbers using commercially available artificial media for the host, and under a wide variety of ambient conditions never inducing diapause in either species. Fourthly, the host and parasite can be collected and reared during almost any time of the year, making the

establishment of laboratory colonies easy and accessible. Lastly, there is no published information on *A. aristoteliae* and its susceptibility or responses to any pesticide.

METHODS AND MATERIALS

General Procedures. Artificial media utilized for rearing and food chain toxicity experiments with nonparasitized hosts, *A. citrana*, and *A. citrana* parasitized by *A. aristoteliae*, was codling moth diet #9370 supplied by Bioserve(R)[1]. Fungicides used were DuPont, benomyl (Benlate)[2] formulated as a 50% wettable powder (50WP) and its principal breakdown product, MBC 98% technical grade. Artificial media was prepared as per Bioserve's instructions, and fungicides were incorporated into the media as reported by Livingston et al. (1978). An exception to the fungicide incorporation procedure was that compounds were added when media reached 35-40°C rather than 45°C.

Preliminary studies were conducted to develop procedures for rearing both hosts and parasites, to determine optimum hosts stages for testing the effects of benomyl on parasitization and to evaluate the effect of feeding regimes and concentrations of benomyl or MBC sublethal to hosts but possibly affecting parasite larval emergence (unpublished data). Third instar *A. citrana* were optimal for experimental evaluation of parasitization by *A. aristoteliae*. Second and third instar *A. citrana* were unaffected by concentrations of 300ppm benomyl or less in their food. This concentration is at the bottom range of recommended field concentration applied for

foliar feeding pests in the field on many crops (0.5-1.0 lbs/ 100 gal = 300-600 ppm). Emergence of *A. aristoteliae* from *A. citrana* larvae, were affected at these same concentration levels of toxicant.

Parasitization of *A. citrana* by *A. aristoteliae* was accomplished by placing 10 third instar larvae into a clear one ounce sting cup fitted with a snap over lid which had a 2 cm diameter hole cut in the center. Host larvae were placed in sting cup with a moistened #2 brush after which the edge of the hole was encircled with honey. Honey prevented host larvae from escaping the chamber and attracted female parasites to the sting cup entrance. A single sting cup was placed in the corner of a communal parasite cage closest to a natural light source for one hour. After exposure, the sting cup with host larvae was removed from the parasite cage and host larvae removed with a soft brush. Head capsule diameters of each host larva were measured prior to their random assignment to treatment combinations (see later discussion). Only third instar larvae, with a head capsule diameter of 0.40-0.56mm (Coop 1982), were used for test evaluations. These were determined using a dissecting microscope fitted with an ocular micrometer.

Four sting cups (10 larvae per cup), as described above, were a treatment replicate and its paired control.

Larvae in each sting cup were exposed to parasites in a serial sequence over a four hr. period (one cup per hour). Larvae from all cups were divided in half to total 20 larvae in each of the treatment and control comparisons. An exception was for tests which required *A. citrana* to feed on treated media prior to exposure to *A. aristoteliae* (see Experiment 1, exposures +,- and +,+). These tests required keeping track of treated and untreated (control) test organisms at all times. Replicates for these tests were obtained by alternately placing cups of treated and untreated *A. citrana* larvae (2 cups each) in the parasite cage. The sequence of cups to be placed in the cage was selected by chance. Test replicates for hosts exposed to parasites were taken on three different days. The size of the parasite colony and the daily activity pattern of the adult parasites permitted only one replicate of parasitized hosts to be obtained per day (with 20 larvae in the experimental and 20 larvae in the control group).

Experimental tests. Experiments included four tests (Table 3) designed to evaluate the impact of benomyl or its metabolite MBC on host larvae exposed to parasites (P), hosts not exposed to parasites (NP), and developing parasites (PA). Day 0 for P and host larvae was the day host larvae were exposed to parasites. and for NP host larvae the first day of the experiment. All concentrations of toxicant used in tests (ppm) were calculated on a wet

Table 3. Test procedures for evaluation of the effects of benomyl and its principal metabolite, MBC, on parasitized and nonparasitized *A. citrana* larvae and the braconid *A. aristoteliae*.

Experiment 1. Effects of standard exposure regimes (300ppm benomyl treated media) on P and NP *A. citrana* larvae and developing *A. aristoteliae* larvae.

Exposure regime 1 (+,-); P and NP host larvae reared on treated media five days prior Day 0 and on untreated media continuously there after.

Exposure regime 2 (-,+); P and NP host larvae reared on treated media only after Day 0.

Exposure regime 3 (+,+); P and NP host larvae reared on treated media five days prior to Day 0 and on treated media continuously there after.

Control (-,-); P and NP host larvae reared on untreated media at all times.

Experiment 2. Effects of different concentrations on P and NP host larvae and developing parasite larvae (000,300,600 & 1200ppm) (-,+) exposure only.

Exposure regime 1 (-,+) 150 ppm: P host larvae only

Exposure regime 2 (-,+) 300 ppm: P and NP host larvae

Exposure regime 3 (-,+) 600 ppm: P and NP host larvae

Exposure regime 4 (-,+) 1200 ppm: P and NP host larvae

Exposure regime 5 (-,-) 000 ppm: P and NP host larvae

Experiment 3. Effects on P hosts and developing parasites of (-,+; 300ppm benomyl) feeding regimes of three durations.

Exposure regime 1 (-,+1); Host larvae were kept on treated media for 1 day after Day 0 and reared on untreated media there after.

Exposure regime 2 (-,+3); Host larvae were kept on treated media for 3 days after Day 0 and reared on untreated media there after.

Exposure regime 3 (-,+5): Host larvae were kept on treated media for 5 days after Day 0 and reared on untreated media there after.

Control (-,-); Host larvae reared on untreated media at all times.

Experiment 4. Effects of MBC 300ppm (-,+) treated media on P hosts and developing parasites.

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media weight basis. All experiments were conducted in an enviromental chamber at a constant temperature regime of 75°F (23.8°C) ±2°F, and photoperiod of 16:8, light:dark.

Experiment 1, the standard exposure regime, was conducted on both P and NP hosts (Table 3). Three host feeding regimes of benomyl laced media (+,-;-,+;+,+: see Table 3 for definition of these designations) of 20 individuals per treatment were replicated 3 times. Each P host replicate was paired to a control (-,-) replicate of 20 individuals each resulting in a total of nine P host control replicates for tests 1,2 and 3 and three for test 4 (Table 4). In Experiments 2 and 3, replications and NP and P host treatments and controls were identical to Experiment 1 (Table 4). Experiment 2 measured the effects of increasing concentrations of benomyl in media on NP hosts, P hosts and developing PAs under treatment conditions (-,+) only. Experiment 3 evaluated the effects of the (-,+) feeding regimes at three time durations of exposure to 300ppm benomyl treated media by P hosts and developing PAs.

Experiment 4 estimated the effects of the metabolite MBC, on P hosts and developing parasites at 300ppm concentration and the (-,+) feeding regime. Test and control replicates (Table 4) were paired as described in

Table 4. Replicate numbers and total population sizes for Experiments 1,2,3,and 4 for evaluation of side effects of benomyl on *A. citrana* and *A. aristoteliae*.

Experiment 1		(-,-)	(+,-)	(-,+)	(+,+)	feeding regime
Standard		(000)	(300)	(300)	(300)	concentrations
exposure	---	---	---	---	---	---
regimes		r=9	r=3	r=3	r=3	replicates
	P^1	---	---	---	---	---
		n=180	n=60	n=60	n=60	total pop.
	---	---	---	---	---	---
		r=3	r=3	r=3	r=3	replicates
	NP^2	---	---	---	---	---
		n=60	n=60	n=60	n=60	total pop.
Experiment 2		(-,-)	(-,+)	(-,+)	(-,+)	(-,+)	feed.reg.
Dosage effects		(000)	(150)	(300)	(600)	(1200)	concen.
	---	---	---	---	---	---	---
		r=9	r=3	r=3	r=3	r=3	reps.
	P	---	---	---	---	---	---
		n=180	n=60	n=60	n=60	n=60	tot. pop.
	---	---	---	---	---	---	---
		r=3	r=3	r=3	r=3	-	reps.
	NP	---	---	---	---	---	---
		n=60	n=60	n=60	n=60	-	tot.pop.
Experiment 3		(-,-)	(-,+1)	(-,+3)	(-,+5)		feed.reg.
Varied (-,+)		(000)	(300)	(300)	(300)		concen.
	---	---	---	---	---	---	---
		r=9	r=3	r=3	r=3		rep. num.
	P	---	---	---	---	---	---
		n=180	n=60	n=60	n=60		tot. pop.
Experiment 4		(-,-)	(-,+)				feed.reg.
Effects of MBC		(000)	(300)				concen.
	---	---	---	---	---	---	---
	P	r=3	r=3	-	-		rep. num.
		---	---	---	---	---	---
		n=60	n=60				tot. pop.
	---	---	---	---	---	---	---

1: P= parasitized hosts
2: NP= nonparasitized hosts

Experiment 1. Adjustments were made for MBC's lower weight to volume as compared to benomyl to make treated media contain 300ppm MBC on a unit weight basis.

The specific factors evaluated in experiments 1-4 (above) were host and parasite survivorship or mortality and time for larval development of hosts and parasites. The survivorship parameter for host and parasite larvae was based on successful pupation. Host mortality included larval death by unknown causes before Day 30, failure to pupate by Day 30 or death resulting from parasite emergence. Those larvae reaching Day 30 alive were measured for head capsule diameter, width at the midpoint and length, then dissected and examined for presence or absence of parasite larvae. Hosts failing to pupate or not succumbing to an emerging parasite larva by day 30 were classified as blocked in development. Estimations of host weight were made from a regression formula developed from a host volume / weight regression relationship (r^2=0.975). Volume was estimated by the formula V = Pi r^2 l, where r = the averaged radius ([Head capsule diameter + width at 0.5 the length] / 4) and length was measured from the tip of the abdomen to the tip of the head capsule. Measurements were made on hosts where movement was minimized and just prior to dissection.

The larval developmental time to pupation for hosts and parasites was calculated from Day 0 to the day of pupation or emergence from host respectively. Observations were made daily.

Data analysis. All non-parasitized host (NP) treatments were evaluated for significant differences ($P < 0.05$) from controls (000 ppm -,-) with an Analysis of Variance (ANOVA), computer software package from NCSS[3](R). Five nonparasitized host treatments with benomyl were compared to unpaired control tests equal in size and number of replicates to a single NP treatment.

In contrast, tests on hosts exposed to parasites (P) were first evaluated for possible block effects. Variation in block effects was principally due to the daily variation in the ability of the parasite colony to parasitize 40 third instar larvae and was manifested in paired controls maintained for each treatment evaluation. The elimination of block effects allowed for testing a simpler ANOVA model, which pooled all 27 P control(-,-) replicates for comparisons with all pesticide treatments. As block effects were determined to be of minor significance, Duncan's Multiple-range tests were used to compare differences among treatments with the 27 pooled controls. Multiple range tests were used to evaluate the effects of treatments alone and the influence of

parasitization and treatments on host and parasite larval development times. Separate evaluations were made for hosts pupating and dying (HPD) and also pupae that eclosed to adult males (HPM) and pupae that eclosed to adult females (HPF).

RESULTS

The Effects of benomyl on nonparasitized host survivorship and development. Host survivorship, measured as host larvae completing pupation (hp), averaged 93% for control (--) NP host larvae (Table 5). No significant difference ($P > 0.05$) in the survivorship response existed between the 300 ppm treatments (+,-; -,+ and +,+) and the control without benomyl, 000 ppm (-,-). With benomyl at 600 and 1200 ppm, however, the average percent of host survivorship decreased to 64 and 35%, respectively (Table 5). A concentration response was evident for decreasing nonparasitized host larval survivorship (NPHLS) and increasing benomyl concentrations over the range of 300 ppm -,+; 600 ppm -,+ and 1200 ppm -,+; $r^2 = 0.98$ (Figure 1). The estimated LC 50 for NP third instar larvae was 806 ppm. This concentration is slightly above the lower recommended field application rate of 0.75 lbs. ai. benomyl per 200 gallons water per acre (=450 ppm) but within the recommended range of 0.5-1.0 lbs ai. per 100-200 gal. water per acre (=300-1200 ppm).

Average times to pupation and multiple-range comparisons among all nonparasitized treatments including three classes of host pupae: those that died as pupa (HPD), those eclosed to males (HPM) and those eclosed to females (HPF) are presented in Table 5 above the dotted line.

Figure 1. Survivorship responses (probits) to the log of benomyl concentration in artificial diet consumed by A. citrana for parasitized host larvae surviving (PHLS) (i.e. those pupating, producing a parasite or blocked in larval development) and nonparasitized host larvae surviving (NPHLS).

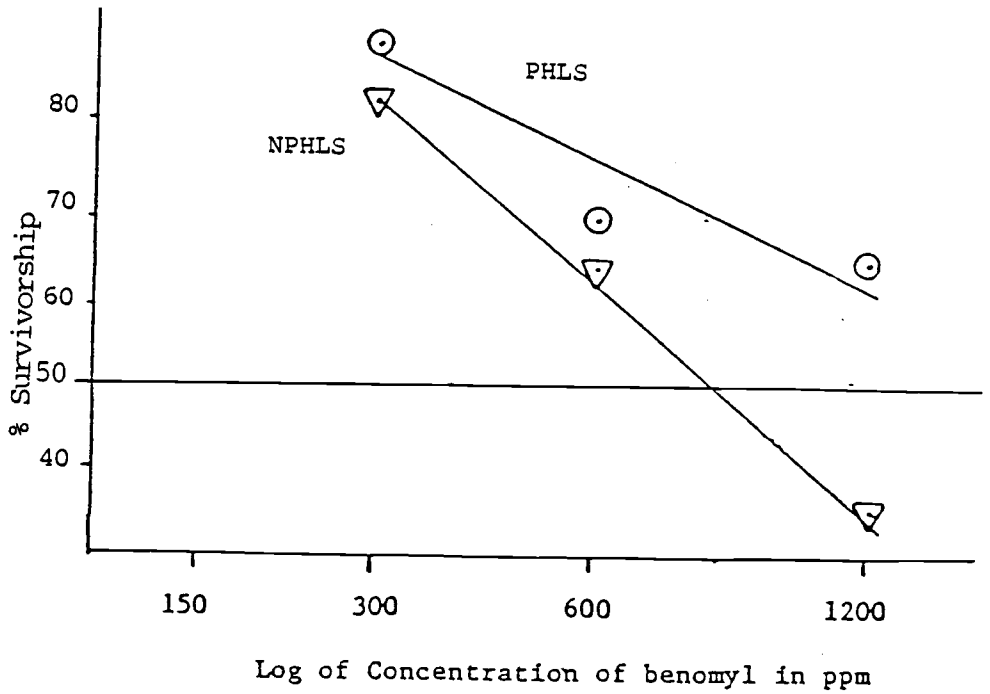


Table 5. Comparisons of the mean % host survivorship and mean number of days to pupation for all treatments of *A. citrana* larvae not exposed to parasites *A. aristoteliae* (NP) above dotted line and exposed to parasites (P) below dotted line.

concentrations (ppm) and exposure regimes[1]	Mean % Host Survivorship[2]	Mean number of days to pupation[2,3] HPD	HPM	HPF
NP 000 -,-	93.0 a	13.9 ab	11.7 ab	14.3 ab
NP 300 +,-	98.0 a	15.8 abc	12.4 abc	15.5 ab
NP 300 -,+ cont.	81.0 a	12.9 a	10.6 a	13.0 a
NP 300 +,+ cont.	94.0 a	16.4 abc	13.6 abcd	16.0 abc
NP 600 -,+ cont.	64.0 b	18.6 cd	14.4 bcd	16.9 bc
NP 1200 -,+ cont.	35.0 cd	17.7 bc	16.1 def	18.6 cd
P 000 -,-	18.0 d	14.4 ab	13.4 abcd	14.4 ab
P 300 +,-	33.3 cd	16.8 abc	14.4 bcde	17.3 bcd
P 300 -,+ 1 day	48.3 bc	29.0 g	19.1 fg	19.6 cdef
P 300 -,+ 3 days	41.7 bc	24.0 efg	19.3 fg	21.1 ef
P 300 -,+ 5 days	53.3 bc	28.5 g	18.2 efg	20.1 def
P 300 -,+ cont.	45.0 bc	24.3 efg	19.1 fg	20.2 cdef
P 300 -,+ MBC cont	48.3 bc	19.3 cdef	15.6 cde	21.2 def
P 300 +,+ cont.	38.3 bc	21.9 cde	15.5 cde	19.3 cdef
P 600 -,+ cont.	28.3 cd	22.9 def	20.5 g	21.5 ef
P 1200 -,+ cont.	5.0 d	25.0 fg	20.0 g	23.0 f

1. A + or - indicates whether or not host larvae fed on benomyl treated media. The (,) represents day 0 of the test, a + before the (,) indicates host larvae fed for 5 days before day 0 and a + after the (,) indicates host larvae fed on treated media after day 0. The durations of feedings on treated media after day 0 were continuous (,+ cont.), for one day (,1 day), three days (,+3 days) or for five days (,+5 days). Exposure regimes are for benomyl except where MBC is noted.

2. Multiple-range coefficients. Mean values within a column and followed by the same letter are not significantly different from each other.

3. Separate comparisons were made for host pupae dying (HPD), pupae eclosing to males (HPM) and pupae eclosing to females (HPF).

Control (-,-) host pupae that died (HPD) (these pupa could not be sexed) showed intermediate developmental times that were in between those for pupating male (HPM) or female (HPF) hosts. Compared to NP control (--), only the NP 600 -,+ and NP 1200 ppm -,+ treatments had longer average times to pupation ($P < 0.05$) for all three pupal catagories examined (HPD, HPM and HPF).

Effects of daily variations in parasitization rates on test results. In test replicates, there was variation in rates of parasitism in controls (-,-) which presumably would have affected interpretation of the differences observed for the test parameters of parasitism rates (pe), host survivorship (hp), blocked hosts (a30) and dissected hosts with or without parasites (dis) among all parasitized host treatment tests (P +,-; -,+1; -,+3; -,+5; -,+; and +,+) (Table 6). To evaluate possible affects on results, two ANOVA models models were compared. The first, a two way ANOVA, included both the fixed factor, treatment, and the random factor, replicates or blocks. It provided a paired comparison of each treatment replicate with its control made on the same day. A second ANOVA model used only the fixed factor, treatments. The F ratio tail probabilities for both models, all test variables measured and results of in Experiments 1-4 treatments are presented in Table 6.

Table 6. Effects benomyl and MBC on hosts *A. citrana* exposed to parasites *A. aristoteliae* and emerging parasites Experiments 1-4.

concentrations (ppm) and exposure regimes[1]	variable[2]	mean % responding[3] control	mean % responding[3] treatment	F-test tail probabilities[4] treatment	F-test tail probabilities[4] block + treatment
300	hp	20.0	33.3	0.94	0.43
+,-	a30	0.0	0.0	1.00	1.00
	dis	0.0	0.0	1.00	1.00
	pe	71.6	60.0	0.71	0.46
300	hp	31.7	48.3	0.21	0.57
-,+1 day	a30	5.0	23.3	0.06	0.28
	dis	0.0	5.0	0.14	0.46
	pe	60.0	13.3	0.005	0.07
300	hp	13.3	41.7	0.07	0.39
-,+3 days	a30	1.7	30.0	0.01	0.14
	dis	0.0	5.0	1.00	1.00
	pe	81.7	15.0	0.01	0.03
300	hp	25.0	53.3	0.09	0.32
-,+5 days	a30	1.7	13.3	0.25	0.67
	dis	6.7	3.3	0.50	0.41
	pe	65.0	15.0	0.01	0.01
300	hp	33.3	45.0	0.35	0.75
-,+	a30	1.7	38.3	0.01	0.02
cont.	dis	1.7	16.7	0.02	0.01
	pe	58.3	10.0	0.01	0.09
300	hp	13.3	48.3	0.02	0.01
-,+ MBC	a30	1.7	38.3	0.001	0.01
cont.	dis	1.7	15.0	0.02	0.11
	pe	81.7	13.3	0.005	0.01
300	hp	16.7	38.3	0.31	0.29
+,+	a30	0.0	33.3	0.001	0.001
cont.	dis	0.0	6.7	0.16	0.47
	pe	80.0	21.7	0.03	0.04
600	hp	5.0	28.3	0.005	0.01
-,+	a30	1.7	43.3	0.001	0.02
cont.	dis	5.0	13.3	0.02	0.34
	pe	85.0	1.7	0.001	0.01

Table 6 continued

concentrations (ppm) and exposure regimes[1]	variable[2]	mean % responding[3] control	mean % responding[3] treatment	F-test tail probabilities[4] treatment	F-test tail probabilities[4] block + treatment
1200	hp	3.3	5.0	0.38	0.50
-,+	a30	5.0	68.3	0.01	0.01
cont.	dis	3.3	25.0	0.02	0.02
	pe	81.7	1.7	0.001	0.01

1. Indicates whether or not host larvae fed on benomyl treated media 5 days before and for how long after day 0 of the test larvae were left on treated media. Exposure regimes are for benomyl except where MBC is noted.

2. Variable codes

Hp = host survivorship measured as percent of larvae pupating per treatment.

a30 = blocked host larvae measured as percent of host larvae per treatment alive and neither pupating or producing a parasite within 30 days of exposure to parasites.

dis = Percent of host larvae per treatment blocked in larval development (A30) dissected and found to contain parasite larva(e).

pe = Percent host larvae per treatment succumbing to an emerging parasite larva, also a measure of parasite survivorship.

3. Mean values are per cent of 60 observations calculated on percent values (Y) where Y = # of observations / 20 for each replicate.

4. Tail probabilities are the result of F ratios calculated on arc sine transformations of the percent values. Actual values are less than reported values. The tail probabilities are for comparisons of treatments (+,-; -,+; +,+) versus paired controls (-,-).

Overall, the two factor (two-way) ANOVA model did not produce many more cases of treatment significance as compared to controls at $P < 0.05$ than did the one factor (one-way) ANOVA model (Table 6). Results of the one-way ANOVAs allowed for the pooling of all 27 replicate controls against which differences among all treatment comparisons could be made using the Duncan's multiple-range test. All results reported hereafter are based on the one-way ANOVA and Duncan's multiple range tests.

Effects of benomyl and parasitization on host survivorship and development. Control host survivorship (000 ppm -,-; hp) after exposure to *A. aristoteliae* averaged 18 % when all 27 test replicates were pooled (Table 5 below dotted line). Multiple-range comparisons indicated that percent host surviorship for the 300 ppm treatments, -,+ 1 day; -,+ 3 days; -,+ 5 days; -,+; +,+ and MBC -,+, were significantly higher than parasitized controls ($P < 0.05$) but that the 300 ppm +,-, 600 ppm and 1200 ppm treatments were not (Table 5). At the highest dosage (1200 ppm; Table 5), host mortality was direct from benomyl as was previously shown for nonparasitized hosts (Table 5 above dotted line). A trend of decreasing host survivorship was evident for increasing concentrations of benomyl (300-1200 ppm).

The time interval for control (-,-) P host larvae to complete development was 14.4 ± 6.2 days for host pupae dying (HPD), 13.4 ± 5.8 days for males (HPM), 14.4 ± 4.2 days for females (HPF) (Table 5). As compared to nonparasitized host larvae (000 ppm -,- NP, Table 5), exposure to parasites alone (000 ppm -,- P) did not affect the average number of days for any category (i.e. HPD, HPM, HPF) of surviving host larvae to reach pupation. All P host benomyl and MBC treatments, except benomyl (300 ppm; +,-), increased the average number of days to pupation for all host categories when compared to NP and P control (-,-) development times (Table 5). Comparisons of common P host versus NP host benomyl treatments for the average number of days to pupation for all three pupal classes showed significant differences only between treatments 300 ppm (-,+), 600 ppm and 1200 ppm (Table 5). There were also general indications that benomyl treatments in parasitized hosts prolonged host pupation as compared to similar treatments within nonparasitized (NP) hosts.

Host development anomalies. Blockage of host larval development was observed only in hosts exposed to parasites (P), occasionally in control and especially in fungicide treatments (Table 7). Observations indicated that if larvae blocked in development were supplied with fresh media, they survived for as long as 120 days after

Table 7. Parasitized *A. citrana* blocked in larval development not pupating or succumbing to a parasite, *A. aristoteliae*, by day 30 (A) and containing parasite larva(e) upon dissection.

concentrations (ppm) and exposure regimes[1]	(A) mean % blocked host values[2,3]	(B) mean % hosts containing parasite larva(e)[2,3]	(C) Proportion blocked hosts with parasite larva(e)[3,4]
000 -,-	2.0 a	1.5 ab	72.5 b
300 +,-	0.0 a	0.0 a	-
300 -,+1 day	23.3 bc	5.0 b	21.5 a
300 -,+3 days	30.0 cd	5.0 b	16.7 a
300 -,+5 days	13.3 b	3.3 ab	24.8 a
300 -,+ cont.	38.3 cd	16.7 c	43.6 a
300 -,+ MBC cont.	38.3 cd	15.0 c	39.2 a
300 +,+ cont.	33.3 cd	6.7 b	20.1 a
600 -,+ cont.	43.3 de	13.3 c	30.7 a
1200 -,+ cont.	68.3 e	25.0 d	36.6 a

1. A + or - indicates whether or not host larvae fed on benomyl treated media. The (,) represents day 0 of the test, a + before the (,) indicates host larvae fed for 5 days before day 0 and a + after the (,) indicates host larvae fed on treated media after day 0. The durations of feedings on treated media after day 0 were continuous (,+ cont.), for one day (,1 day), three days (,+3 days) or for five days (,+5 days). Exposure regimes are for benomyl except where MBC is noted.

2: Mean values are per cent of 60 observations calculated on percent values (Y) where Y = (total # of observations)/60.

3: Multiple-range coefficients were calculated on arcsine transformed data, and mean values within a column and followed by the same letter are not significantly different from each other.

4: Proportional values (C) are calculated from the ratio of blocked hosts (A) to those dissected and found to contain parasite larvae (B). C = A / B.

exposure to parasites. Control (-,-) P host population had an observed rate of blocked host larval development of 2.0 % or 11 out of 540 hosts (Table 7). MBC and all benomyl treatments except (300 ppm; +,-), resulted in significantly higher percentages ($P < 0.05$) of blocked hosts than P host (-,-) groups (Table 7). A trend of an increased blocked hosts relative to increasing levels of benomyl at concentrations 300 ppm, 600 ppm and 1200 ppm was also evident (Table 7, Column A).

Comparing the percent of blocked larvae per 60 individuals per treatment, fungicide treatments having the greatest percent of blocked hosts also showed the greatest percent of hosts containing parasite larvae upon dissection (Table 7, Column B). However, all fungicide treatments contained a relatively constant proportion of blocked hosts with parasite larva(e) (variable in number across treatments: Table 7, Column C). Blocked control (-,-) hosts contained the highest proportion of detectable parasite larvae per blocked hosts and significantly ($P < 0.05$) higher than any fungicide treatment (Table 7, Column C). However as noted earlier the number of blocked hosts observed in controls over all replicates was very few. No discrimination in difference between live and dead parasite larvae were made in these tests due to the difficulties in determining the condition of 1^{st} instars.

Regulation of host development and host metamorphosis by *A. aristoteliae.* Evidence for regulation of host growth and development (i.e., reductions in weight gain and inhibition of larval metamorphosis to pupa) by the parasite was readily evident in P control (-,-) hosts that eventually succumbed to a parasite larva. Benomyl treated blocked hosts were highly variable in size on Day 30 independent of whether they contained parasite larva(e) upon dissection. This suggested that regulation of host development was due to factors other than the presence of parasite larva(e) alone. To determine if parasite larva affected blocked host growth (i.e., day 30 weights) and to what extent host growth was regulated in blocked hosts, weights of male and female penultimate NP host larvae reared on untreated (000 ppm) media and blocked P host larvae with and without parasites were compared (Table 8). Day 30 weights for blocked male larvae were not significantly smaller than for penultimate NP male larvae on Day 10. Blocked female larvae with parasite larva were significantly smaller than either blocked females without parasites or NP penultimate females. Since penultimate NP male larvae normally achieve weights only half those of females, differences in degree of host regulation by parasites in blocked hosts might not be as evident for male as for female hosts.

Table 8. Weights of normal and blocked *A. citrana* larvae, exposed to parasites, *A. aristoteliae*.

Larval sex	normal or blocked hosts[1]	dissected parasite larva(e)	larval host weights[2,3]	
male	B	yes	10.6 mg	a
male	B	no	11.0 mg	a
female	B	yes	16.2 mg	a
female	B	no	30.3 mg	b
male	N	-	17.8 mg	a
female	N	-	39.4 mg	b

1: B = Host larvae which were blocked in development and reared on various concentrations of benomyl (0-1200 ppm); N = host larvae which were reared untreated diet and pupated.

2: Comparisons are based on larval sex and presence or absence of parasites, mean values within a column and followed by the same letter are not significantly different from each other.

3: P host weights (Y) were estimated Day 30 weights from host volume measurements (X) using the regression formula $Y = 1.0358X - 0.048$ ($r^2 = 0.957$). NP host weights were taken just prior to pupation on Day 11 and represent mature host larval weights.

Effects of MBC and benomyl treatments on the developing parasite larvae. Parasite larval survivorship was reduced by all fungicide treatments except benomyl (+,-) 300 ppm (Table 9). No differences were found among the 300 ppm post exposure treatments (-,+1 day, -,+3 days, -,+5 days, -,+ and +,+). The intermediate parasite survivorship of the 300 ppm +,+ treatment suggested that prior exposure of the host larva to benomyl did not influence the survival ability of the parasite. A trend of decreasing parasite emergence was evident over the concentrations of 150-600 ppm. At the 600 and 1200 ppm concentrations larval parasite emergence was almost completely inhibited. No consistent trend was evident for the effect of fungicide treatments on parasite larval developmental times.

Table 9. Parasite larvae, *A. aristoteliae* emerging from hosts which were reared on artificial diets, untreated (-,-) or treated with the benomyl or MBC.

concentration	exposure regime[1]	mean value[2]	multiple-range coefficient[3]
000 ppm	-,-	72.5	a
150 ppm	-,+ cont.	41.7	a
300 ppm	+,-	60.0	a
300 ppm	-,+ 1 day	13.3	bc
300 ppm	-,+ 3 days	15.0	bc
300 ppm	-,+ 5 days	15.0	bc
300 ppm	-,+ cont.	10.0	bc
300 ppm	-,+ MBC cont.	13.3	bc
300 ppm	+,+ cont.	21.7	b
600 ppm	-,+ cont.	1.7	c
1200 ppm	-,+ cont.	1.7	c

1. A + or - indicates whether or not host larvae fed on benomyl treated media. The (,) represents day 0 of the test, a + before the (,) indicates host larvae fed for 5 days before day 0 and a + after the (,) indicates host larvae fed on treated media after day 0. The durations of feedings on treated media after day 0 were continuous (,+ cont.), for one day (,1 day), three days (,+3 days) or for five days (,+5 days). Exposure regimes are for benomyl except where MBC is noted.

2. Mean values (Y) are per cent values of the total treatment observations (60).

3. Multiple-range coefficients were calculated on arcsine transformed data, and mean values within a column and followed by the same letter are not significantly different from each other.

The effects of benomyl on host and parasite survivorship to adulthood. Multiple range tests indicated that host survivorship to adulthood was significantly ($P < 0.05$) lower in the P host control (-,-) and P host 1200 ppm (-,+) treatments (Table 10). The highest host survivorship occurred in the NP host treatments 000 ppm, 300 ppm and 600 ppm treatments (Table 10). Of the P host treatments only the 300 ppm -,+ 1 day and -,+ 5 days had significantly higher ($P < 0.05$) host survivorship than Control (-,-) P host treatments, although there were trends of higher survivorship in all 300 ppm treatments (Table 10). Parasite survivorship was significantly greater ($P < 0.05$) in the control (-,-) and 300 ppm (+,-) treatments compared to all other treatments (Table 10), again confirming the lack of effect on parasites of hosts exposed only to benomyl prior to parasitization. Host and parasite survivorship from pupation to adulthood was relatively constant across all treatments (compare Tables 5 and 9 with Table 10).

Table 10. The effect of benomyl and MBC treatments on larval survivorship to adulthood for hosts, *A. citrana*, exposed to parasites (P), not exposed to parasites (NP) and parasites *A. aristoteliae* .

concentrations (ppm) and exposure regime[1]	Mean % adult host survivorship[2,3]	Mean % adult parasite survivorship[2,3]
NP 000 -,-	68.3 a	-
NP 300 -,+ cont.	63.3 ab	-
NP 600 -,+ cont.	51.7 ab	-
NP 1200 -,+ cont.	28.3 cde	-
P 000 -,-	13.3 ef	61.7 a
P 300 +,-	25.0 de	50.0 a
P 300 -,+ 1 day	45.0 abcd	11.1 b
P 300 -,+ 3 days	36.7 abcde	10.0 b
P 300 -,+ 5 days	46.6 abcd	15.0 b
P 300 -,+ cont.	33.3 bcde	10.0 b
P 300 -,+ MBC cont.	36.6 abcde	5.0 b
P 300 +,+ cont.	20.0 de	13.3 b
P 600 -,+ cont.	16.7 de	1.7 b
P 1200 -,+ cont.	1.7 f	0.0 b

1. A + or - indicates whether or not host larvae fed on benomyl treated media. The (,) represents day 0 of the test, a + before the (,) indicates host larvae fed for 5 days before day 0 and a + after the (,) indicates host larvae fed on treated media after day 0. The durations of feedings on treated media after day 0 were continuous (,+ cont.), for one day (,1 day), three days (,+3 days) or for five days (,+5 days). Exposure regimes are for benomyl except where MBC is noted.

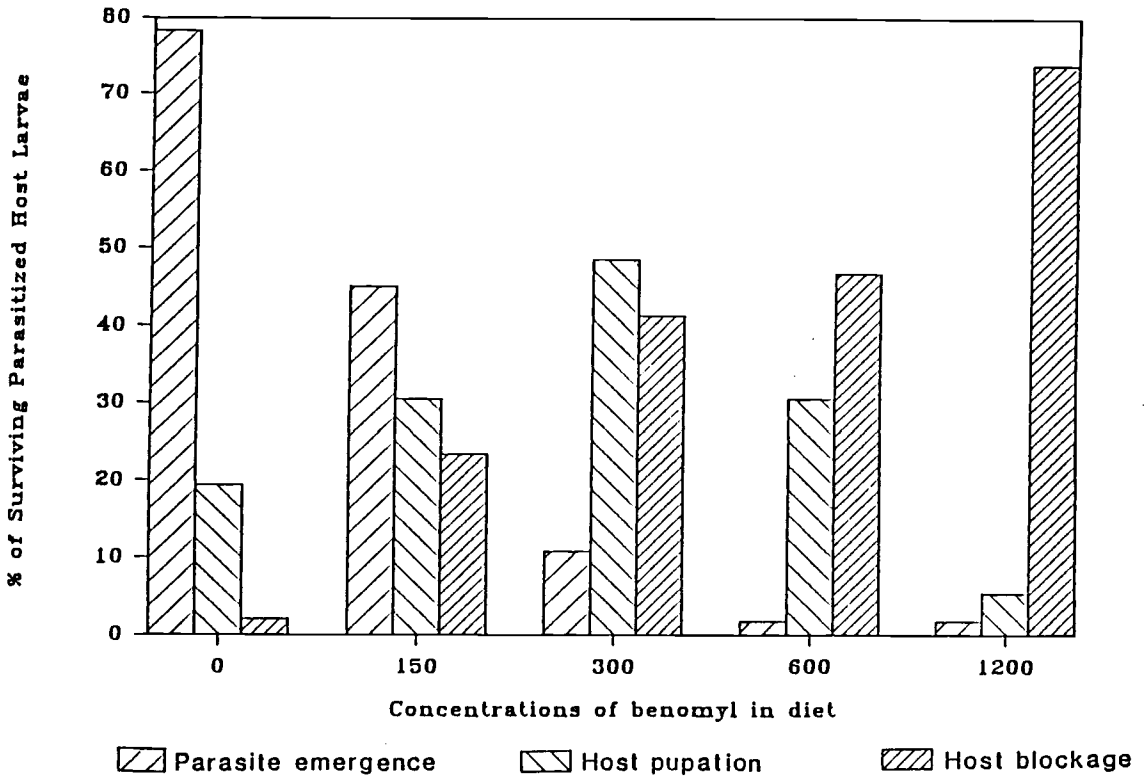
2. Mean values (Y) are percent values of the total treatment observations (60).

3. Multiple-range coefficients were calculated on arcsine transformed data, and mean values within a column and followed by the same letter are not significantly different from each other.

Relative toxicity of benomyl to parasitized versus nonparasitized host larvae. In these tests, nonparasitized larvae either died from the benomyl or they pupated. However parasitized host larvae, if they survived the benomyl treatments, fell into three catagories; those pupating, those producing a parasite or those surviving as larvae blocked in development. Comparisons among common treatments (300 ppm -,+; 600 ppm -,+ and 1200 ppm -,+; Figure 1) of larval survivorship for nonparasitized hosts (NPHLS) versus parasitized hosts for all three catagories mentioned above (PHLS), indicated that parasitized host larvae were less susceptible than nonparasitized host larvae to the concentrations tested. Probit values of survivorship regressed to the log of benomyl concentrations (300-1200 ppm) showed a linear response (r^2=0.88) and LC50 value of 2151 ppm for P host larvae. As noted earlier, nonparasitized host larvae showed a linear response (r^2=0.99) to benomyl and LC50 of 806 ppm (Figure 1).

If one separates out the survivorship percentages for each subclass of PHLS hosts, i.e., those pupating, those producing parasites or those blocked in larval development in relation to benomyl concentrations, the impact on populations as they relate to pest levels and biological controls can be better identified (Figure 2). Percentages

Figure 2. Percentages of subclasses of parasitized *A. citrana* larvae surviving benomyl treatments (see PHLS Figure 1).



of parasite emergence and hosts blocked in development decrease and increase, linearly in relation to increasing benomyl concentrations, respectively. Host pupation percentages increase from ca. 20% in controls to peak near 50% at 300 ppm and decline thereafter at 600 and 1200 ppm.

DISCUSSION

At the rates tested in this study, benomyl and its principal breakdown product MBC affected both nonparasitized and parasitized *A. citrana* larvae and the developing endoparasite *A. aristoteliae*. These effects were both direct and indirect (i.e., via the other organism) and involved the complex interactions of the host and parasite. The range of effects on these species was diverse and included direct mortality in the host, sublethal effects on host development via the parasite, blockage of host development, parasite mortality via the food chain and a chemotherapeutic effect of ridding hosts of parasites. The complexities of these interactions are discussed below.

Several aspects of benomyl's behavior in the host were established in this study. Benomyl was not toxic to nonparasitized hosts at levels $\leq$ 300 ppm and was not retained in the host at levels toxic to the developing parasite egg(s) if consumption of 300 ppm benomyl treated media stopped before parasite eggs were layed. Host feedings on 300 ppm benomyl for 24 hrs. after exposure to parasites produced effects equivalent to continuous host feedings on benomyl indicating that developing parasite embryos were absorbing lethal amounts very rapidly. Both

observations indicate benomyl's transient nature in the host.

Increases in parasitized host survivorship at the 300 ppm -,+ treatment represented a chemotherapeutic effect of benomyl in ridding parasitized hosts of *A. aristoteliae*. However parasite emergence was decreased at all concentrations tested. Comparisons of LC_{50} values for parasites to parasitized or unparasitized hosts indicated a selectivity ratio difference of 1:8-20. These ratios are generally less than those commonly found for other hymenopteran:lepidopteran larval forms (Croft and Mullin 1984 and Teague et al. 1985). These results indicate that a rather narrow window for chemotherapeutic effects was present in these laboratory tests. However, for other species the window may be much wider. These studies raise the question of how often or to what extent, similar chemotherapeutic effects occur in the field considering the dynamics of declining residues on foliage and the timing of parasite attack following sprays.

Increased host larvae blocked in development and the overall low levels of parasite larvae found in dissected hosts indicated that factors other than the presence of parasite larvae were contributing to the blockage of host development. Death of the parasite prior to egg eclosion seemed to be involved in determining whether parasitized

hosts would be blocked or would finish larval development and pupate. This was evidenced by data for 24 hour host feedings on the toxicant which indicated near equivalent effects of blockage of host development as did continuous feeding tests on benomyl or MBC.

It is possible that release of teratocytes upon eclosion of parasite egg(s) combined with ovipositional fluids (i.e., poison and calyx gland fluids) and viral particles associated with these fluids were responsible for the observed increased percentages of blocked hosts regardless of subsequent parasite development. Guillot and Vinson (1972) and Ables and Vinson (1981) noted the role of calyx gland and poison gland fluids of *Chelonus insularis* and *Cardiochiles nigriceps* in reducing host weight gains and delaying development of *H. virescens* snd *S. ornithogalli*. Baculovirus particles have been identified in the calyx fluids of a large number of braconid and ichnuemonid endoparasites and and are presumably present in *A. aristoteliae*. These virus particles have been speculated to contribute to the regulation of host growth and development (Stoltz and Vinson 1980). Although these studies report similar host growth responses to those observed in this study they do not report the total inhibition of host development that occurred here.

Teratocytes, unicellular forms derived from the embryonic membrane of parasite eggs (Salt 1968; Sluss 1968), were observed in this study in all dissected hosts with parasites and many without. Teratocytes have been shown to live and grow in the absense of parasite larvae (Vinson 1970). When Vinson injected teratocytes of *C. nigiceps* into unparasitized *H. virescens* larvae, host growth was similar to that of parasitized larvae, however, as in the above experiments with calyx and poison gland fluids, pupation was only delayed. Vinson and coworkers have demonstrated that while alone, ovipositional fluids and associated viral particles or teratocytes are capable of reducing weight gain they are not capable of preventing host pupation. Consistent with the above research, the findings of this study suggest that completion of parasitized *A. citrana* larval development was only possible if parasite eggs were prevented from eclosing. This was reflected here as increases in the percentages of host pupating at the 300 ppm benomyl. If *A. aristoteliae* eggs eclosed, the combined interaction of released teratocyte cells, baculovirus particles and ovipositional fluids resulted in the complete blockage of host development regardless of the subsequent fate of the parasite larva. The apparent ovicidal activity of benomyl as observed in these tests has been documented in other studies for several predatory and pest mite species (Delp

and Klopping 1968; Binns 1969; Stafford and Fukushima 1971; Spaddafora and Lindiquist 1972; and Nakashima and Croft 1974).

Comparative LC50 data for nonparasitized and parasitized larvae including those pupating, producing a parasite or blocked in larval development indicated that parasitized forms were less susceptible to benomyl than nonparasitized forms. It is speculated that this may be due to the reduced consumption of food by parasitized hosts as reflected by the delays in onset of pupation for hosts surviving parasitization and reduced growth rates observed for blocked hosts.

Results similar to those observed in these tests have been previously reported for other host-parasite associations. Teague et al. (1985) noted that parasitized second instar *H. zea, S. exiguae* and *P. includens* remained in second and third stadia for 20-30 days following parasitization if reared on benomyl treated diets. However unlike in Teague's study, parasitized, blocked *A. citrana* continued to grow and molt, some achieving the Day 11 normal penultimate larval weight by Day 30 following parasitization.

As observed in these studies, the use of benomyl, other benzimidazole compounds or even more specific chemical agents may represent new noninvasive measures

(e.g., as compared to microsurgery, an invasive technique) for manipulating parasitized hosts in the laboratory. Benomyl's transient nature in this study seemed to give it the properties of a surgical technique by eliminating the parasite in the host at a very specific stage of development (i.e. egg). A comparative evaluation of the side effects of these types of measures (i.e., chemical) would have to be made in relation to other measures of endoparasite manipulation before these techniques could be recommended. These types of chemical methods, however, may prove useful in basic studies of host parasitoid interactions in the future.

A final question relates to the possible effects that benomyl or other similar acting compounds which are sublethal to a host but lethal to a developing endoparasite might have under field conditions. As noted, the answer would depend on the concentrations of toxicant sprayed, the residue dynamics on the host food, behavior in the host and the timing of parasite oviposition in the host. Additional studies of the dynamics of these systems in nature are needed to evaluate the magnitude of these effects in the field for a variety of lepidopteran-endoparasitoid life systems. It is possible that these types of responses of natural enemies to pesticides in nature are far more common than we have perceived.

ENDNOTES

1: Bioserve Inc.
French Town, New Jersey 08825

2: Benlate is a proprietory product of
E.I. dupont de Nemoirs and Co.
Wilmington, Delaware. 19899.

3: NCSS, Number Cruncher Statistical System.
Copyright 1984,1985 by Dr. J.L. Hintze
865 East 400 North
Kaysville, Utah 84037

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